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A tumor suppressive DNA translocase named FANCM

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Abstract

FANCM is named after Fanconi anemia (FA) complement group M. The clinical symptoms of Fanconi anemia include congenital abnormalities, pancytopenia and cancer proneness. However, recent studies reveal that bi-allelic inactivation of FANCM does not cause the constellation of Fanconi anemia symptoms, but predisposes patients to cancer and infertility. FANCM is a tumour suppressor gene that encodes a conserved and structure-specific DNA translocase. It controls the outcome of homologous recombination and facilitates DNA replication across a variety of natural and chemically-induced obstacles. This review details our current understanding of FANCM as a facilitator of the cellular functions of caretaker proteins, including Fanconi anemia, Bloom syndrome and Ataxia telangiectasia and RAD3 related proteins, which collectively ensure the maintenance of chromosome stability during DNA replication.

Introduction

FANCM has its roots in ancient forms of life. It is orthologous to Hef (Helicase-associated endonuclease for fork-structured DNA), a protein of the Archaeobacteria kingdom. Hef was originally discovered by Ishino's team in a screen for *Pyrococcus furiosus* proteins that would enhance the resolution of Holliday junctions by Hjc (Holliday junction resolvase) (Komori et al., 2002). FANCM was identified by mass spectrometry as a factor associated with the multi-protein Fanconi anemia ubiquitin ligase complex (Meetei et al., 2005). FANCM stands for Fanconi anemia group M protein, but current evidence indicates that FANCM deficiency does not cause Fanconi anemia. Fanconi anemia (FA) is an inherited chromosomal instability disorder associated with congenital abnormalities, severe bone marrow failure and a marked predisposition to developing acute myeloid leukemia in childhood and, later in life, solid tumors of the head, neck and gynecological system (Alter et al., 2003; Kottemann and Smogorzewska, 2013; Tischkowitz and Hodgson, 2003). The diagnosis of FA is confirmed when in addition to conspicuous clinical symptoms, patient cells are hypersensitive to interstrand DNA crosslinking agents and exhibit a high frequency of broken and radial chromosomes (Auerbach, 1993). FANCM was dubbed a FA protein because bi-allelic mutations in *FANCM* were discovered in patient EUFA867, the sister of a patient diagnosed with FA, and because her cells were more sensitive than normal cells to DNA crosslinking agents (Meetei et al., 2005). Unlike her brother who carried bi-allelic mutations in the *bona fide* FA gene *FANCA*, patient EUFA867 did not exhibit visible symptoms of FA (Singh et al., 2009). Furthermore, the hypersensitivity of her cells (EUFA867) to interstrand DNA crosslinking agents could not be rescued with a cDNA encoding FANCM (Meetei et al., 2005). The cells were later found to carry, in addition to mutations in *FANCM*, the same bi-allelic *FANCA* mutations than her sibling who was presenting overt FA symptoms (Singh et al., 2009). Thus, among these two siblings, the clinical symptoms of the carrier of bi-allelic mutations in both *FANCA* and *FANCM* were less severe than the symptoms of the sibling carrying mutations in *FANCA* only.

Consistent with the absence of FA symptoms associated with *FANCM* mutations, no evidence of bone marrow failure was observed in Finns carrying homozygous *FANCM* loss of function mutations (Lim et al., 2014). Several individuals carrying bi-allelic non-sense mutations in *FANCM* were identified recently (Bogliolo et al., 2018; Catucci et al., 2018). These patients did not exhibit congenital malformations and bone marrow failure but developed early onset cancers and were highly

sensitive to chemotherapies (Bogliolo et al., 2018; Catucci et al., 2018). A male was diagnosed with B-cell precursor lymphoblastic leukemia and two siblings developed squamous carcinomas in the mouth and neck (Bogliolo et al., 2018). Cells derived from these patients exhibited chromosomal instability and were sensitive to interstrand DNA crosslinking agents. These cellular phenotypes were corrected with the *FANCM* cDNA (Bogliolo et al., 2018). A contemporaneous study reported five cases of homozygous loss-of-function mutations in *FANCM* associated with breast cancer and early menopause (Catucci et al., 2018). *FANCM* knockout mice also have an increased incidence of cancers (Bakker et al., 2009). Another study reported homozygous-truncating *FANCM* mutations in a Finnish family with two patients with primary ovarian insufficiency, or premature menopause, which could result from meiotic defects (Fouquet et al., 2017). Pathogenic variants of *FANCM* are also associated with male infertility due to a reduced number or the complete absence of spermatozoa (Kasak et al., 2018; Yin et al., 2018). In sum, FANCM loss-of-function is associated with infertility and cancer predisposition, but not with the collection of clinical symptoms that defines Fanconi anemia.

FANCM is in the 15th position among the most frequently mutated DNA damage response genes across various cancer types (Knijnenburg et al., 2018). Significant tumor specific loss of heterozygosity occurs in the *FANCM* gene (Lu et al., 2015). Carriers of *FANCM* mutations have been identified among patients with breast and ovarian cancers in diverse populations (Dicks et al., 2017; Kiiski et al., 2014; Kiiski et al., 2017; Neidhardt et al., 2017; Nguyen-Dumont et al., 2018; Peterlongo et al., 2015; Silvestri et al., 2018; Stafford et al., 2017). These findings suggest that FANCM should be included in diagnostic gene panel testing for breast and ovarian cancer susceptibility.

Structure and biochemical activity

FANCM family proteins include an amino terminal DEAH helicase domain, a carboxyl terminal ERCC4 nuclease domain, and an intervening sequence of variable length (Figure 1). Human FANCM, however, does not exhibit endonuclease nor helicase activity, but has DNA-dependent ATPase activity and translocates along double-stranded DNA.

Studies of *FANCM* orthologs in model organisms, such as *Schizosaccharomyces pombe* (*Fml1*), *Saccharomyces cerevisiae* (*Mph1*), *Arabidopsis thaliana* and *Drosophila melanogaster* (*FANCM*), have provided essential insights into the function of the FANCM family of proteins. FANCM has multiple and genetically separable functions in DNA damage responses, including helicase independent and carboxy-terminal independent function (Romero et

al., 2016). For an exhaustive description of the FANCM family and associated biochemical activities, we recommend the reading of excellent reviews by Whitby (Whitby, 2010) and Xue (Xue et al., 2015).

The amino terminus

Meetei et al. first showed that FANCM translocates along double-stranded DNA (Meetei et al., 2005). The authors found that FANCM can displace a triplex forming oligonucleotide from duplex DNA (Meetei et al., 2005). A few months before the identification of FANCM as a FA core complex-associated protein, Nishino et al. had published the crystal structure of the helicase domain of *Pyrococcus furiosus* Hef (Nishino et al., 2005). The structure revealed an Hef-specific domain inserted between the two conserved RecA-like helicase domains (Nishino et al., 2005). This positively charged insertion is similar to the “thumb” domain of polymerases and critical for the recognition and for the processing of branched DNA structures (Nishino et al., 2005). Nishino et al. noted that FANCM, at that time known as hypothetical protein KIAA1596, was orthologous to Hef and suggested that KIAA1596 may also process branched DNA structures. Like Hef, FANCM has an insertion between helicase motifs III and IV (Ishino et al., 2014). A crystal structure of the helicase domain of FANCM is necessary to confirm if this insertion encodes a “thumb” domain that confers functional specificity. FANCM indeed binds with exquisite specificity and affinity to branched DNA molecules such as model replication forks and four-way junctions, with apparent dissociation constants of about 0.1 nM (Gari et al., 2008b). FANCM can couple ATP hydrolysis with the migration of the branch point of four-way junctions and of model replication forks through kilobases of DNA (Gari et al., 2008a; Gari et al., 2008b; Xue et al., 2008; Yan et al., 2010). Branch point translocation at forks induces reannealing of the “parental” DNA strands, which leads to the extrusion of a fourth duplex arm at the branch point, a process called fork reversal. Like FANCM, the *S. pombe* and *S. cerevisiae* homologs Fml and Mph1 catalyze replication fork reversal in vitro (Sun et al., 2008; Zheng et al., 2011).

FANCM forms a pentameric complex with a heterodimer of MHF1/MHF2 (or CENP-S/X) that is structurally similar to histone H3-H4 (Singh et al., 2010; Tao et al., 2012; Yan et al., 2010; Zhao et al., 2014). The FANCM-MHF complex is conserved in plants, yeasts and silkworm (Bhattacharjee et al., 2013; Girard et al., 2014; Sugahara et al., 2014; Yan et al., 2010). The integrity of the FANCM-MHF complex is required for the suppression of meiotic crossovers in plants (Girard et al., 2014), and for the maintenance of genome stability in budding yeast,

fission yeast and in cancer cell lines (Bhattacharjee et al., 2013; Singh et al., 2010; Yan et al., 2010). The FANCM-MHF complex is stabilized by a zinc atom at the interface of MHF and the MHF-interaction domain of FANCM (Fox et al., 2014). One study suggests that upon binding to FANCM, the DNA binding surface of MHF is altered in a way that increases its affinity for Holliday junctions (Fox et al., 2014). Another study concludes that MHF binds branched DNA molecules more strongly than duplex DNA as it engages two duplex arms (Zhao et al., 2014). Both studies show that MHF stimulates the branch migration activity of FANCM (Figure 1) (Fox et al., 2014; Yan et al., 2010; Zhao et al., 2014).

The carboxy-terminus

The carboxy terminus of FANCM is homologous to the ERCC4 nuclease domain of the XPF/MUS81 family of heterodimeric endonucleases, but it lacks essential residues required for nuclease activity (Ciccia et al., 2007). The ERCC4 domain of FANCM associates with FAAP24 and forms a rigid S shaped structure that binds double-stranded DNA (Ciccia et al., 2007; Coulthard et al., 2013). Electron microscopy images indicate that the pseudo nuclease domain of FANCM is in close proximity to its amino terminal translocase domain, suggesting that both domains bind cooperatively to DNA replication structures (Coulthard et al., 2013). No homologs of FAAP24 have been identified in *A. thaliana*, *S. cerevisiae*, *S. pombe*, *D. melanogaster* or *C. elegans* (Girard et al., 2014). FANCM and FAAP24 have both common and independent functions in human cells (Figure 1) (Wang et al., 2013). Only a sub-fraction of total FAAP24 is bound to FANCM (Ciccia et al., 2007) and FAAP24 is not essential for FANCM branch migration activity.

The intervening sequence

Between the conserved amino- and carboxy-termini of FANCM, an intervening sequence spanning amino acid 599 to amino acid 1841 is predicted to be unstructured or intrinsically disordered (Figure 1) (Byers III et al., 2017). The sequence between the amino terminal helicase domain and the carboxy-terminal nuclease domain of archeal Hef protein is also intrinsically disordered, as revealed by circular dichroism, nuclear magnetic resonance and atomic force microscopy (Ishino et al., 2014). This intrinsically disordered domain may fold upon interaction with a diversity of protein partners, including PCNA and a RecJ-like protein (Ishino et al., 2014). On the other hand, the intrinsically disordered intervening sequence of *S. cerevisiae* Mph1 functions as a protein-based/prion-like epigenetic element that promotes resistance to genotoxic stress by self-templating protein conformations (Byers III et al., 2017). Mph1 has

been identified in an unbiased screen of the yeast proteome for proteins that can induce heritable traits by transient overexpression and propagate them long after their expression level has returned to normal (Chakrabortee et al., 2016). Mph1 overexpression exerts toxicity in strains that have inherited the Mph1 prion, a characteristic hallmark of prion proteins. Intriguingly, overexpression of the intrinsically disordered region of human FANCM is also toxic to yeast strains that have inherited the Mph1 prion (Byers III et al., 2017). These observations suggest that the large intervening region in FANCM may act as a prion involved in protein-mediated epigenetic inheritance.

The functions of FANCM

A suppressor of crossovers

Available evidence indicates that the branch point translocase activity of FANCM family of proteins promotes non-crossover recombination in mitosis and in meiosis. *S. pombe Fml1* and *D. melanogaster FANCM* suppress crossover formation during both mitotic and meiotic recombination (Kuo et al., 2014; Lorenz et al., 2012; Nandi and Whitby, 2012; Sun et al., 2008; Zafar et al., 2017), while in *S. cerevisiae*, the channeling by Mph1 of recombination intermediates into a non-crossover pathway has been demonstrated during mitosis (Prakash et al., 2009). *A. thaliana FANCM* was identified in a screen for mutations induced by ethylmethane sulfonate that restore fertility defects caused by low crossover frequency (Crismani et al., 2012). FANCM also suppresses meiotic crossovers in *Brassica crops* (Blary et al., 2018). Reciprocal exchanges of DNA in sister chromatids (SCEs) are suppressed by FANCM in chicken DT40 cells (Rosado et al., 2009), in primary mouse embryonic fibroblasts (Bakker et al., 2009), and in human cancer cell lines (Deans and West, 2009; Wang et al., 2013). The elevated incidence of cancers associated with *FANCM* loss of function may be caused by an increased rate of promiscuous recombination events leading to loss of heterozygosity (LOH).

During the repair of double-stranded DNA breaks by homologous recombination, DNA ends are resected to yield 3' single-stranded DNA tails required for the assembly of the RAD51 nucleoprotein filament. The RAD51 recombinase promotes an exchange of strands between the broken ends and the homologous DNA template that yields a displacement loop (D-loop), as the broken strand displaces its homolog and pairs with the complementary strand of the intact duplex (Figure 2). The invading 3' strand then primes DNA repair synthesis using the intact strand as a template. During synthesis-dependent strand

annealing (SDSA), the D-loop structure is dissociated after DNA repair synthesis and the elongated DNA strand can now reanneal with its original complementary strand (Figure 2). The remaining gaps are eventually filled in and ligated to yield an intact duplex. Biochemical evidence indicates that FANCM family of protein Fml1, Mph1 and FANCM can all dismantle D-loop structures in an ATPase dependent manner (Gari et al., 2008a; Prakash et al., 2009; Sun et al., 2008). This biochemical activity suggests that FANCM family proteins suppress crossovers via the dissolution of D-loops, thereby promoting synthesis-dependent strand annealing (SDSA) (Figure 2).

A facilitator of BLM's function

FANCM associates with the Bloom's syndrome (BS) nuclear complex (Deans and West, 2009; Hoadley et al., 2012; Meetei et al., 2003). The complex includes the RecQ helicase BLM, Topoisomerase III β , RMI1 and RMI2 (Wu et al., 2000; Xu et al., 2008). The BLM helicase can promote the branch migration of Holliday junctions (Karow et al., 2000). Together with the decatenase Topoisomerase III β , BLM catalyzes the dissolution of recombination intermediates linked by a double Holliday junction (Figure 2) (Wu and Hickson, 2003). This suggests that BLM prevents sister chromatid exchanges through the dissolution of replication-associated recombinant intermediates. Double Holliday junction dissolution by BLM-TOPOIII β separates the recombination molecules without reciprocal exchange of genetic material (Figure 2). BS cells are characterized by an increased frequency of sister chromatid exchanges, a diagnostic feature recapitulated in BLM-deficient mice (Luo et al., 2000).

FANCM interacts with RMI and Topoisomerase III β via an evolutionary conserved motif called MM2, located between the amino terminal helicase domain and the C-terminal ERCC4 domain (Figure 1) (Deans and West, 2009). The MM2 peptide interfaces with the RMI complex (RMI1/RMI2) via "knobs-into-holes" hydrophobic contacts (Hoadley et al., 2012). FANCM facilitates the targeting of BLM to specific DNA damage sites. FANCM requirement for the concentration of BLM into nuclear foci remains to be clarified. One study used stable FANCM siRNA cell lines and showed that FANCM facilitates BLM foci formation in response to cellular treatments with mitomycin C (MMC) or camptothecin (CPT) (Deans et al., 2018). BLM foci were not restored after complementation with a siRNA resistant cDNA encoding a BLM interaction-dead mutants of FANCM, indicating that FANCM targets BLM to MMC-stalled forks (Deans and West, 2009). By contrast, the formation of BLM foci induced by MMC was minimally affected in HCT116

FANCM^{-/-} knockout cells obtained by homologous replacement using a recombinant adeno-associated virus (rAAV)-based construct delivery system (Wang et al., 2013). This apparent discrepancy illustrates that the production of stable cell lines is associated with the inherent risk of selecting specific clones that have adapted to the loss of an important function. Stable knockdown or knockout cells may diverge significantly from the initial population of cells and express compensatory activities. In DT40 cells, the helicase activity of BLM is required for the formation of FANCM foci induced by cellular exposure to MMC (Ling et al., 2016). (Deans and West, 2009; Ling et al., 2016; Wang et al., 2013) Interestingly, both FANCM and BLM are synthetic viable when the stability of the FA ubiquitin ligase complex is compromised (Moder et al., 2017). *FANCC* encodes a subunit of the FA ubiquitin ligase complex (Figure 3). The suppression of FANCC destabilizes the complex and renders cells sensitive to interstrand DNA crosslinking agents. In the near haploid cells HAP1, the loss of either *FANCM* or *BLM* attenuates the sensitivity of *FANCC* knockout cells to MMC (Moder et al., 2017), suggesting that both proteins participate in a pathway that is toxic when the FA ubiquitin ligase complex is disrupted (Moder et al., 2017). Alternatively, since the screen for synthetic viability is based on gene knockout, selection for cells expressing a compensatory activity may have occurred.

Thus, BLM and FANCM are linked physically and functionally, but additional studies are necessary to define precisely the nature of the functional interaction between FANCM and BLM, in particular because BLM and FANCM exert independent functions in human physiology. The clinical symptoms of BS patients are different from the symptoms of patients carrying biallelic mutations in *FANCM*. BS individuals have distinct facial features, such as a narrow face and prominent nose, sun-sensitive facial erythema, and a marked predisposition to the full range of cancers observed in the general population (Hanada and Hickson, 2007).

A facilitator of the FA pathway

FA proteins are required for the repair of interstrand DNA crosslinks (ICLs) induced by chemotherapeutic drugs (Sasaki and Tonomura, 1973). The mechanism of interstrand DNA crosslinks repair is now understood in considerable details (Raschle et al., 2008). Replication-coupled repair of an ICL requires the convergence of two forks on the ICL, forming a X-shaped DNA structure (Figure 3) (Zhang et al., 2015). The replicative helicase is unloaded (Fullbright et al., 2016; Long et al., 2014), and one of the two converging replication forks regresses into a four-way junction

(Amunugama et al., 2018). The motor protein that catalyzes the reversal of replication fork structures at an ICL remains to be defined. The ICL is unhooked by XPF (FANCC)/ERCC1 and bypassed by the REV1-Pol zeta complex (Budzowska et al., 2015). ICL unhooking yields a double-strand DNA break that is repaired by homologous recombination (Long et al., 2011). The FA ubiquitin ligase complex monoubiquitinates DNA-bound FANCD2 and FANCI (van Twest et al., 2016). The conjugation of a ubiquitin moiety to FANCD2 is necessary for the recruitment of XPF (FANCC) / ERCC1 and of SLX4 (FANCP) to unhook the ICL (Figure 3) (Klein Douwel et al., 2014; Yamamoto et al., 2011).

FANCM interacts with the FANCF subunit of the FA complex via its evolutionally conserved motif MM1 located between the amino- and carboxy-termini (Figure 1) (Deans and West, 2009). The MM1 motif in FANCM is required for MMC-induced FANCD2 monoubiquitination, FANCD2 foci formation and MMC resistance (Figure 1) (Deans and West, 2009). These observations suggest that FANCM anchors the FA ubiquitin ligase complex at ICL stalled replication forks to promote FANCD2 monoubiquitination and ICL unhooking (Figure 3) (Deans and West, 2009). Unlike other subunits of the FA ubiquitin ligase complex, however, FANCM is not essential for the monoubiquitination of FANCD2 (Bakker et al., 2009; Meetei et al., 2005; Mosedale et al., 2005; Singh et al., 2009). FANCM-deficient cells retain the capacity to monoubiquitinate FANCD2, albeit less efficiently. In fact, the majority of FANCM-MHF does not associate with the FA ubiquitin ligase complex in human cells (Yan et al., 2010). This explains why patients that carry bi-allelic inactivation of *FANCM* do not exhibit the characteristic symptoms of Fanconi anemia. Yet, a partial defect in FANCD2 monoubiquitination most likely contributes to the hypersensitivity of FANCM deficient cells to ICLs. In fact, a complex relationship exists between FANCM and the FA ubiquitin ligase complex. The knockout of *FANCM* attenuates mitomycin C-induced apoptosis in avian DT40 cells that lack the FANCC subunit of the FA ubiquitin ligase complex (Mosedale et al., 2005). *FANCM* was also identified in a genome-wide CRISPR/Cas9 screen for synthetic viable interactions with *FANCC* in the near haploid cell line HAP1 (Moder et al., 2017). One possibility is that FANCM activity commits cells to a FA-dependent ICL-repair pathway, whereas suppression of FANCM in cells lacking FANCC may open the way for an alternative ICL tolerance pathway. We discuss below the role of FANCM in replication traverse across an ICL (Huang et al., 2013), which yields the X-shaped DNA structure required for the initiation of replication-coupled and FA-dependent repair of ICLs (Zhang et al., 2015).

A facilitator of ATR signaling

FANCM facilitates ATR/Chk1 signaling (Collis et al., 2007) (Schwab et al., 2010) (Huang et al., 2010). Collis et al provided evidence that FANCM promotes checkpoint signaling in a manner that depends on its translocase activity but independently of the FA ubiquitin ligase complex (Figure 1) (Collis et al., 2008). FANCM appears to facilitate ATR activation in multiple ways. First, FANCM and FAAP24 interact with Telomere length regulation TEL2 homolog, a protein alternatively named (HCLK2), which stands for human homolog of *Caenorhabditis elegans* biological clock protein CLK-2 (Collis et al., 2008). TEL2/HCLK2 associates with ATR and Chk1, promotes ATR/Chk1 signaling and prevents unscheduled proteasomal degradation of Chk1 (Collis et al., 2007). In fact, TEL2/HCLK2 functions as a co-chaperone of HSP90 that is specifically required for the stability of the PI3K-related kinases ATM, ATR, DNA-PKcs, and mTOR (Takai et al., 2007; Takai et al., 2010). TEL2/HCLK2 notably mediates the association of ATR with ATRIP (Takai et al., 2010). We do not know if the role of FANCM in ATR signaling is linked with the chaperoning function of TEL2/HCLK2. Second, Schwab et al. have provided evidence that FANCM facilitates ATR activation via the retention of its activator TopBP1 in chromatin (Schwab et al., 2010). Third, Huang et al. showed that FANCM/FAAP24 promotes ATR activation through RPA foci formation at forks stalled by interstrand DNA crosslinks (Huang et al., 2010). A key step in ATR activation is the formation of RPA-covered single-stranded DNA, which recruits and increases the local concentration of ATRIP-ATR at DNA damage sites (Zou and Elledge, 2003). Stretches of single-stranded DNA typically accumulate at replication forks as a result of helicase and DNA polymerases uncoupling. It is unclear whether and how RPA-coated single-strand DNA would form at interstrand DNA crosslinks that prevent DNA strand separation. Indeed, cellular treatment with mitomycin C does not induce the massive accumulation of single-strand DNA that is observed in cells exposed to hydroxyurea or UV (Huang et al., 2010). The latter study shows that the formation of RPA foci in response to treatment with MMC is dependent on the FANCM/FAAP24 complex and on the binding of FAAP24 to DNA (Huang et al., 2010). The FANCM/FAAP24 complex may yield short single-stranded DNA regions that are not detectable by immunofluorescence staining, or recruit RPA at ICL-stalled forks, directly or indirectly, via protein-protein interactions. (Huang et al., 2010)

In contrast to the above-mentioned studies, which showed that depletion of FANCM by RNA interference attenuates ATR signaling, FANCM^{-/-}

knockout HCT116 cells do not exhibit ATR signaling defects (Wang et al., 2013). In this model system, only FAAP24 but not FANCM is required for ATR signaling (Wang et al., 2013). The absence of ATR signaling defects in FANCM^{-/-} HCT116 cells suggests that cells can adapt to the permanent loss of FANCM, but not of FAAP24. By contrast, the transient and acute depletion of FANCM by siRNA molecules appears to reduce the strength of the ATR signal (Collis et al., 2008) (Huang et al., 2010). Collectively, these data indicate that FANCM facilitates but is not essential for ATR activation. Reciprocally, ATR facilitates the function of FANCM via FANCM phosphorylation on serine 1045 (Singh et al., 2013). Cells expressing the S1045A FANCM mutant are hypersensitive to MMC, fail to accumulate FANCM at sites of interstrand DNA crosslinks and exhibit ATR/Chk1 signaling and G2/M checkpoint defects (Singh et al., 2013).

A facilitator of DNA replication

Accumulating evidence indicates that FANCM ensures the progression of replication forks across difficult to replicate regions and across DNA lesions induced by chemotherapeutic drugs.

Replication traverse of interstrand DNA crosslinks.

Interstrand DNA crosslinks (ICLs) are extremely toxic for DNA replication and transcription because the lesions prevent the separation of DNA strands (Deans and West, 2011). Seidman and colleagues have provided evidence that the replication machinery can move through an ICL (Figure 4) (Huang et al., 2013). The authors used a DNA fiber labeling methodology and trimethylpsoralen (TMP) linked to digoxigenin to visualize at the single molecule level and simultaneously DNA replication tracks and ICLs by fluorescence microscopy. Upon exposure to UVA, TMP forms interstrand DNA crosslinks. Remarkably, replication tracts containing digoxigenin TMP were observed, contrary to the common assumption that an ICL represents an impenetrable block to the progression of replication forks. In this study, the translocase activity of FANCM was essential for the traverse of psoralen crosslinks by replication forks, but not important for DNA replication across DNA lesions induced by angelicin, a molecule related to psoralen that only forms mono-adducts (Huang et al., 2013). How exactly FANCM promotes replication traverse is not yet fully understood. The DNA binding and the activity of the replicative helicase MCM2-7 is controlled by a ATP-dependent “gate” at the MCM2-MCM5 interface, which is maintained in a closed configuration by Cdc45 and GINS (Costa et al., 2011). The traverse of an ICLs most likely involves major structural modifications of the replicative helicase. FANCM may promote the passage of

MCM2-7 in a double-stranded DNA-encircling configuration through the ICL. Replication traverse would yield the X-shaped DNA structure required to initiate replication-coupled repair of DNA crosslinks (Zhang et al., 2015). Like its archaeal homolog Hef (Ishino et al., 2014), FANCM is endowed with a PCNA-interacting protein (PIP) motif at its amino terminus (Figure 1) (Rohleder et al., 2016). The PIP motif is required for FANCM to promote replication traverse across ICLs. FANCM also cooperates functionally with the Bloom's syndrome complex to replicate crosslinked DNA (Figure 4) (Ling et al., 2016). The treatment of DT40 cells with inhibitors of DNA replication induces the accumulation of FANCM within distinct nuclear foci that co-localize with FANCD2 and BLM. These foci presumably reflect nuclear sites of replication fork stalling. The recruitment of FANCM to stalled forks is dependent on its intrinsic DNA translocase activity, on FAAP24, on its association with the Bloom's syndrome complex, on BLM helicase activity, and on its phosphorylation by the replication checkpoint kinase ATR, but is independent of the FA ubiquitin ligase complex (Ling et al., 2016).

Tolerance of DNA replication stress. In addition to the rescue of replication forks stalled by interstrand DNA crosslinks, FANCM exerts a general role in replication stress tolerance. Unlike Fanconi anemia cells, FANCM deficient cells are also hypersensitive to hydroxyurea, to aphidicolin, to UV irradiation and to the topoisomerase I inhibitor camptothecin (Kelsall et al., 2012; Schwab et al., 2010; Singh et al., 2009). FANCM promotes the resumption of DNA synthesis after fork stalling by camptothecin (Luke-Glaser et al., 2010; Schwab et al., 2010). The recovery of DNA synthesis after treatment with camptothecin is dependent on the DNA translocase activity of FANCM (Luke-Glaser et al., 2010). In the absence of any exogenous source of DNA replication stress, FANCM prevents the stochastic arrest of replication forks and the spontaneous accumulation of single-stranded DNA in HeLa cells (Luke-Glaser et al., 2010).

FANCM deficient cells are also sensitive to PARP1 inhibitors (Stoepker et al., 2015). Therefore, patients carrying biallelic FANCM-mutations are probably hypersensitive to PARP1 inhibitors. The utility of PARP1 inhibitors, however, may extend to patients with tumor-specific loss of heterozygosity in the *FANCM* gene.

Protection against replication interference by RNA-DNA hybrids. Transcription-associated DNA-RNA hybrid structures called R-loops represent an important endogenous source of interference with DNA replication (Castellano-Pozo et al., 2013; Gan et al., 2011; Tuduri et al., 2009). R-loops

accumulate when the maturation of pre-mRNA is delayed or upon the build-up of negative supercoiling (Li and Manley, 2005). DNA-RNA hybrids are tightly connected with chromatin compaction, which, in turn, may interfere with the process of DNA replication (Castellano-Pozo et al., 2013). Fanconi anemia proteins and FANCM attenuate DNA replication stress induced by transcription interference, and FANCM can dismantle R-loops *in vitro* (Deans et al., 2018; Schwab et al., 2015). The yeast FANCM homolog Mph1 also attenuates DNA replication stress induced by RNA-DNA hybrids (Lafuente-Barquero et al., 2017). But the activity of Mph1 is controlled by the cohesin-related SMC (structural maintenance of chromosome) 5/6 complex to prevent the formation of toxic structures at replication forks stalled by RNA-DNA hybrids (Lafuente-Barquero et al., 2017). *In vitro*, SMC5/6 inhibits the replication fork reversal activity of Mph1 (Xue et al., 2014). Is transcription interference overcome through replication fork reversal, disruption of RNA-DNA hybrid structures, via the remodeling of nucleoprotein complexes or through another mechanism? Further studies are necessary to clarify how exactly the FANCM family of motor proteins prevent DNA replication stress caused by interference with transcription in cells.

Replication of ALT telomeres. ALT (Alternative Lengthening of Telomeres) is a mechanism that involves homologous recombination and allows human tumors and immortalized cell lines to maintain telomere length independently of telomerase. The sub-telomeric/telomeric region is intrinsically difficult to replicate because this region is rich in guanines, poor in replication origins, and forms RNA-DNA hybrid structures with the non-coding RNA TERRA (Azzalin et al., 2007; Sfeir et al., 2009). Suppression of FANCM or either one of its associated proteins MHF1/2 or FAAP24 induces DNA replication stress in cells that depend on the ALT pathway to maintain telomeres (Pan et al., 2017). FANCM deficiency induces the formation of ALT-associated promyelocytic leukemia (PML) bodies that co-localize with BLM and with markers of DNA replication stress (Pan et al., 2017). The absence of FANCM induces the formation of BLM foci (Pan et al., 2017). So, in ALT cells, the relationship between FANCM and BLM is reversed compared to cells exposed to mitomycin C (MMC) (Deans and West, 2009). In ALT cells that lack FANCM, BLM and BRCA1 act epistatically to promote DNA end resection and to stimulate homologous recombination at ALT telomeres. In sum, FANCM exhibits synthetic lethal interaction with BLM and BRCA1 during DNA replication of ALT telomeres (Pan et al., 2017).

Protection of chromosome fragile sites.

Chromosomal fragile sites (CFSs) are hypersensitive to DNA replication inhibitors and hotspots for chromosomal rearrangements in cancers. Replication forks pause and collapse preferentially at poly(dA:dT) tracts in response to treatment with hydroxyurea (Tubbs et al., 2018). A specific role for FANCM in the protection of CFS stability was revealed using a CFS-derived AT-rich and hairpin forming DNA sequence inserted into a EGFP-based homologous recombination reporter (Wang et al., 2018). In this assay, the DNA translocase activity of FANCM along with its associated proteins FAAP24 and MHF1/2 suppress mitotic recombination and attenuate oncogene-induced fragility of the common chromosome fragile site FRA16D, independently of the FA ubiquitin ligase complex. RAD52 is required for mitotic recombination at CFS-derived AT-rich sequences and is synthetic lethal with FANCM (Wang et al., 2018). These observations designate RAD52 as a potential therapeutic target for FANCM-deficient tumors.

Concluding remarks

In all the organisms studied so far, the FANCM family of motor proteins promotes the separation of recombination intermediates into non-crossover products. An excessive number of crossovers may cause segregation problems during meiosis (Fernandes et al., 2018), and underlie the fertility defects in FANCM-deficient individuals. Less clear is how FANCM suppresses tumors. The first answer that comes into mind is that FANCM prevents cancer through protection against loss of heterozygosity caused by mitotic crossing over. Yet, FANCM associates with a network of DNA caretaker proteins that ensure DNA replication and genome integrity. It has been difficult to integrate the activity of FANCM in a simple model because FANCM facilitates a variety of chromosomal maintenance pathways, including the FA pathway, without being essential for any of them. FANCM is a structure-specific DNA translocase that moves three-way and four-way junctions that mimic the branch point of replication forks, R-loops and Holliday junctions, yet we do not know exactly the nature of the nucleic acid structure(s) targeted by FANCM in cells. The translocase activity of FANCM is required for the resumption of DNA synthesis after inhibition of topoisomerase I with camptothecin and for replication traverse of interstrand DNA crosslinks. It remains to be defined how exactly FANCM ensures fork progression across obstacles that are very different in nature. A precise definition of the cellular substrate(s) and action mechanism(s) of FANCM is necessary and perhaps

sufficient to explain most of its functions. FANCM may also diminish the formation of obstructions to the replisome. Potential roles for FANCM in the displacement of DNA-bound proteins and in the remodelling of nucleoprotein complexes remain to be explored and may provide further insights into how FANCM facilitates chromosome maintenance pathways. With the growing realization that FANCM mutations are implicated in the development of cancers in the general population, it becomes clear that a better understanding of how the structure-specific DNA translocase FANCM maintains the integrity of our chromosomes is an important topic for human health.

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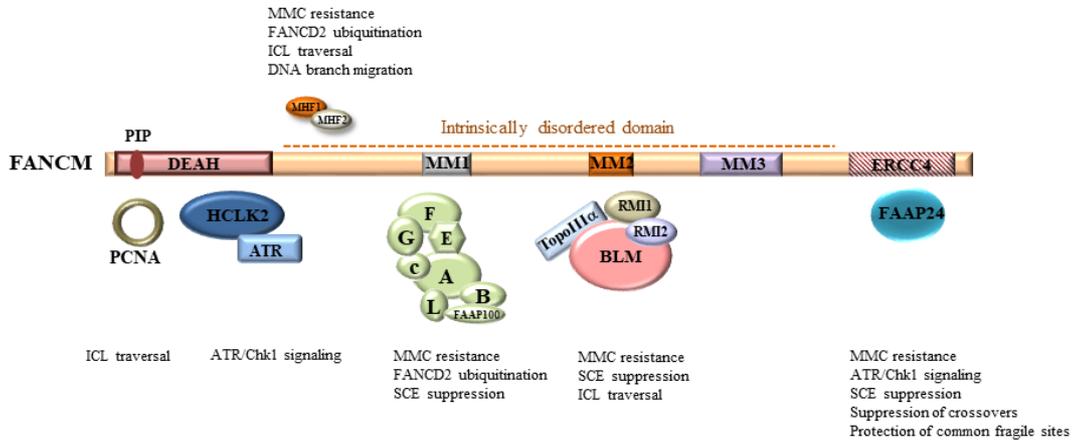


Figure 1

Figure 1. Schematic illustration of FANCM domains organization, partner proteins and biochemical functions. FANCM includes a PCNA-interacting protein (PIP) box, a DEAH box helicase domain and a pseudo ERCC4 nuclease domain separated by an intrinsically disordered intervening sequence that includes the evolutionary conserved FANCM motifs MM1-3. FANCM facilitates a variety of processes required for the maintenance of chromosome stability. FANCM interacting partners and associated biological functions are indicated.

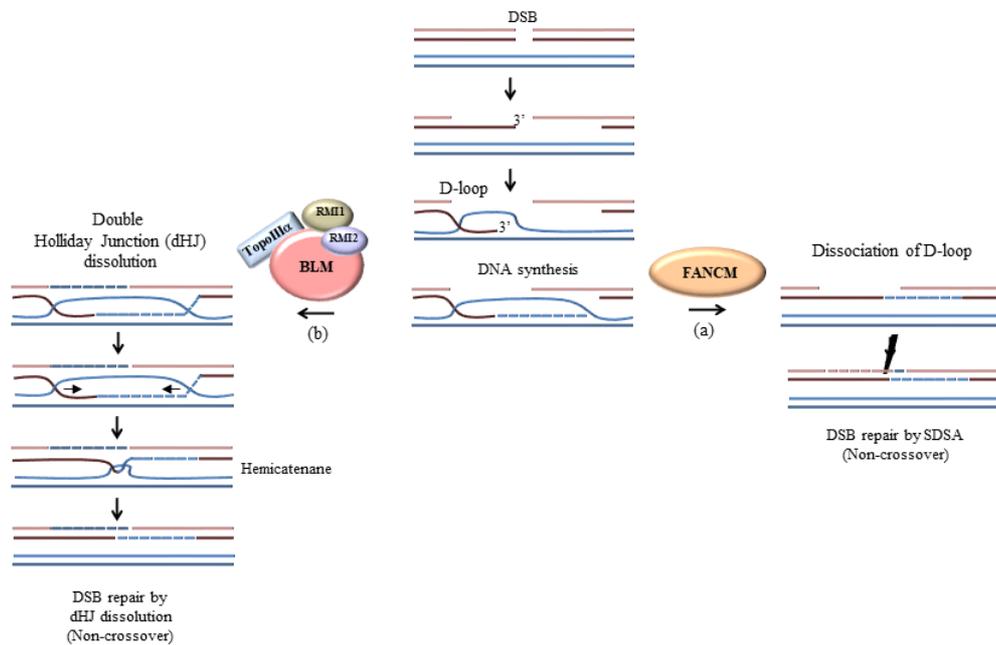
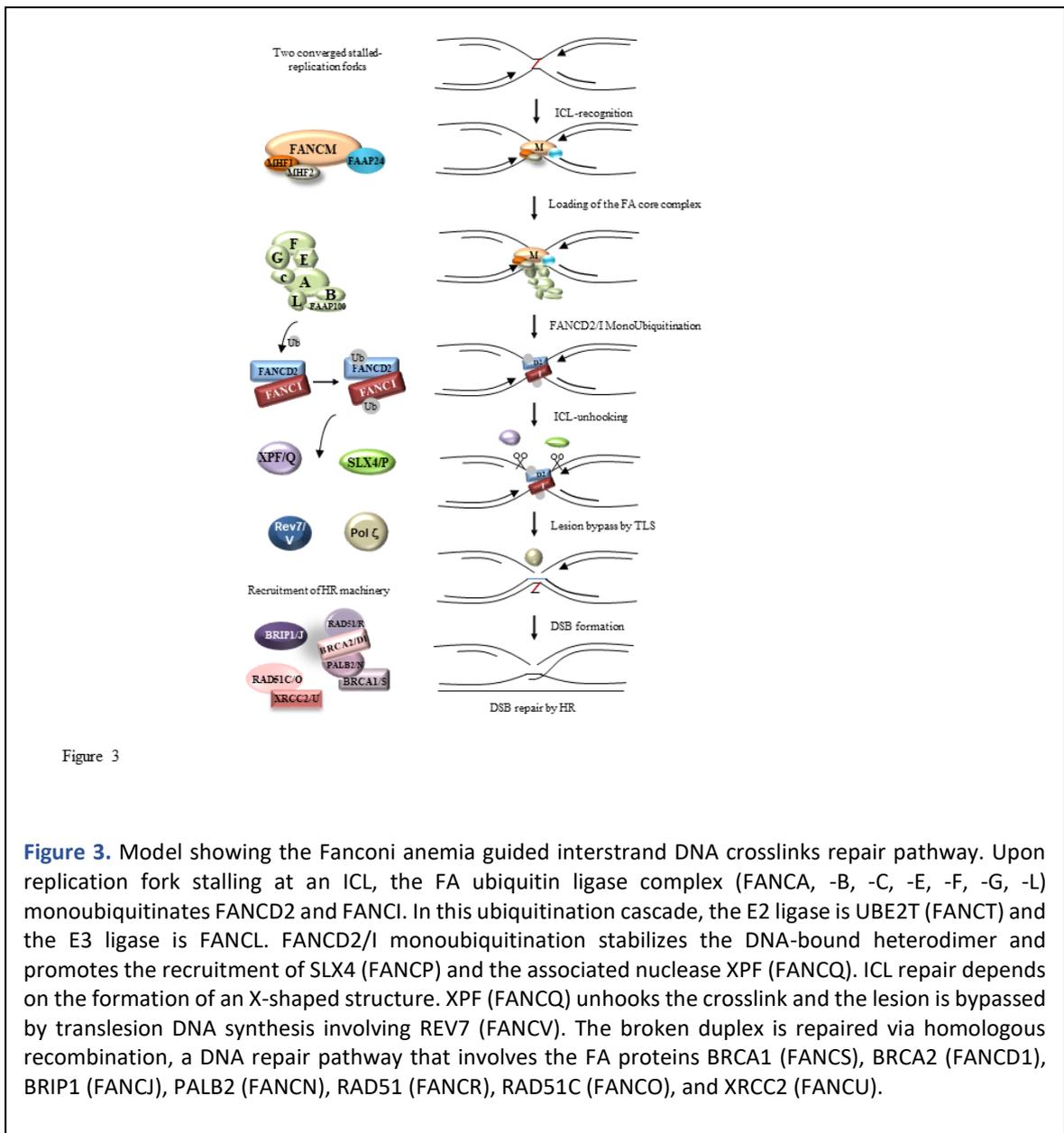


Figure 2

Figure 2. Model showing the homologous recombination sub-pathways driven by BLM and FANCM. Dashed lines indicates newly synthesized DNA. A displacement loop (D-loop) is formed after invasion of the homologous duplex by the 3' single-stranded DNA tail, which primes DNA repair synthesis. (a) FANCM catalyzes the disruption of the D-loop before Holliday junction formation. The extended DNA strand can now anneal with the complementary strand. The repair process is completed by gap filling and ligation. This pathway is called synthesis-dependent strand annealing (SDSA) and yields non-crossover products. (b) BLM promotes the separation of recombining molecules after the formation of a double Holliday junction structure. BLM branch migrates the Holliday junctions to form a hemicatenane. The intertwined molecules are decatenated by Topoisomerase III α . The dissolution of double Holliday junctions yields non-crossover products.



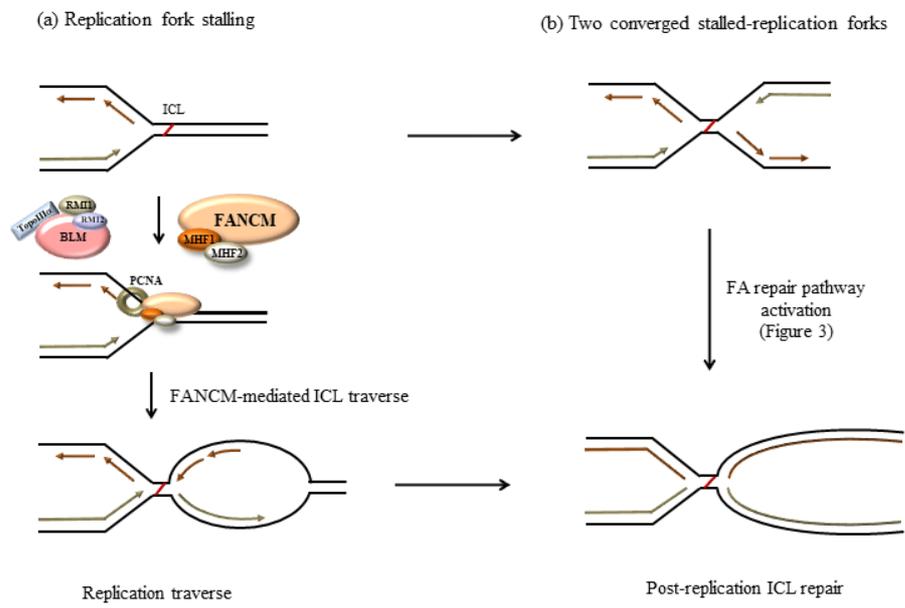


Figure 4

Figure 4. Model showing the formation of X-shaped DNA structures required to initiate replication-coupled interstrand DNA crosslink repair. (a) FANCM and its associated DNA-binding partners MHF1/MHF2 associate with PCNA and promote replication traverse of an ICL in cooperation with BLM. (b) The resumption of DNA synthesis past the ICL yields an X-shaped DNA structure.